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Hydrostatic Pressure Alters the Time Course of GTP[S] Binding to G Proteins in Brain Membranes from Two Congeneric Marine Fishes

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Abstract. The effects of hydrostatic pressure on the receptor-stimulated exchange of guanosine triphosphate (GTP) for guanosine diphosphate (GDP) on the α subunit of G proteins were studied in two congeneric marine teleost fishes that differ in their depths of distribution. The poorly hydrolyzable GTP analog [^{35}S]guanosine 5′-[γ-thio]triphosphate ([^{35}S]GTP[S]) was used to monitor the modulation of signal transduction by the A_1 adenosine receptor agonist N^6-R-(phenylisopropyl)adenosine (R-PIA) in brain membranes of the scorpaenids *Sebastolobus alascanus* and *S. altivelis*. The maximal binding (B_{max}) and dissociation constant (K_d) values, determined from equilibrium binding isotherms at atmospheric pressure (5°C), were similar in the two species. The B_{max} values for these species are much lower than literature values for mammalian brain tissue (25°C); however, the K_d values of the teleost and mammalian G proteins are similar. The EC_{50} values for the A_1 adenosine receptor agonist R-PIA were similar in the two species. Hydrostatic pressure of 204 atm altered the binding of [^{35}S]GTP[S]; basal [^{35}S]GTP[S] binding decreased 25%. The A_1 adenosine receptor agonist R-PIA and the muscarinic cholinergic receptor agonist carbamyl choline stimulated [^{35}S]GTP[S] binding at 1 and 204 atm. At atmospheric pressure the half-time ($t_{1/2}$) of [^{35}S]GTP[S] binding differed between the two species. The GTP[S] on rate (k_{on}) is larger in the shallower-living *S. alascanus*. Increased hydrostatic pressure altered the time course, decreasing the $t_{1/2}$ in both species. The pressures that elicit this change in the time course differ between the species. However, interpolating over the range of *in situ* pressures the species experience, the values are similar in the two species. The guanyl nucleotide binding properties of the G protein α subunits appear to be conserved at the environmental temperatures and pressures the species experience.

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Introduction

The high hydrostatic pressures characteristic of the deep ocean significantly influence guanine-nucleotide-binding protein (G protein)-coupled signal transduction systems (Siebenaller and Murray, 1995). Because of the large number and ubiquity of G protein-coupled signaling complexes, the effects of pressure on such systems will have an important role in shaping the evolution of signal transduction systems in marine species and may play a part in determining the bathymetric distribution of species.

G proteins couple a diverse superfamily of cell surface receptor proteins, characterized by seven membrane-spanning regions, to a variety of effector elements, such as adenylyl cyclase, ion channels, and phospholipases (Spiegel *et al.*, 1994). The subunits of the heterotrimeric G proteins are designated α, β, and γ. The classes of G proteins, such as G_i and G_s, which inhibit and stimulate adenylyl cyclase, respectively, and G_o, a common G protein in brain tissue that may be coupled to Ca^{++} channels and phospholipase C, are defined by the α subunit type (Gilman, 1994).

Receptors with bound agonist interact with heterotrimeric G proteins, promoting the binding of GTP in exchange for GDP on the α subunit. The binding of GTP evokes a conformational change that results in the dissociation of the G protein into $\alpha \cdot$ GTP and a $\beta\gamma$ dimer (Coleman *et al.*, 1994; Wall *et al.*, 1995). The activated $\alpha \cdot$ GTP subunit and the $\beta\gamma$ dimer interact with the target enzyme or ion channel. Signaling is terminated by the hydrolysis of bound GTP by

the intrinsic GTPase activity of the α subunit and the subsequent reassociation of the α and $\beta\gamma$ subunits (Gilman, 1994; Mixon *et al.*, 1995).

Guanyl nucleotide binding is important in the pressure-sensitivity of signal transduction (Murray and Siebenaller, 1993; Siebenaller and Murray, 1994a, b). The present study was designed to test the hypothesis that alteration of guanyl nucleotide binding contributes to the perturbation of transmembrane signaling by hydrostatic pressure. To directly examine one of the initial steps of G protein activation, the binding of GTP, we used the GTP analog guanosine 5′-[γ-thio]triphosphate (GTP[S]). This analog is not hydrolyzed by the GTPase activity of the G protein α subunit (Wieland and Jakobs, 1994). The effects of pressure on the activation of G proteins were studied by measuring the binding of [^{35}S]GTP[S] to the G protein α subunit. The binding of this analog of GTP reflects the GDP-GTP exchange reaction, which is stimulated by receptor agonists.

We have used the A_1 adenosine-G_i-adenylyl cyclase signaling complex as a representative G protein-coupled receptor system to study the influence of environmental factors (Murray and Siebenaller, 1987; Siebenaller and Murray, 1988, 1990, 1995). This system was chosen because it is amenable to the use of frozen tissues, and because of the array of pharmacological tools available to probe the system (Siebenaller and Murray, 1994c). This receptor is found in brain tissue of vertebrates but has not been detected in central nervous tissue of the arthropods or molluscs tested (Siebenaller and Murray, 1986). Adenosine is a potent physiologic regulator with central nervous, cardiac, and peripheral effects (Bruns, 1990; Palmer and Stiles, 1995; Sundin and Nilsson, 1996). Agonist occupation of the A_1 adenosine receptor results in the negative modulation of adenylyl cyclase (Daly *et al.*, 1981).

We have focused on the A_1 adenosine-G_i-adenylyl cyclase signaling complex in brain tissue of two closely related scorpaenid fish species. These fishes have been employed as a model system to study adaptation to hydrostatic pressure. The congeners *Sebastolobus alascanus* and *S. altivelis*, co-occur geographically (Miller and Lea, 1976; Lauth *et al.*, 1997), are genetically close (Siebenaller, 1978), have similar life histories (Moser, 1974), and experience similar temperatures (Hubbs, 1926; Siebenaller, 1984); but they have different patterns of depth distribution (Hubbs, 1926; Siebenaller and Somero, 1978; Wakefield and Smith, 1990; Lauth *et al.*, 1997) and hence encounter different hydrostatic pressures. Pressure increases 1 atm (= 101,325 Pa = 1 bar) for every 10-m depth increase in the ocean (Saunders and Fofonoff, 1976). Demersal *S. alascanus* adults are commonly found between 100 and 850 m and have a maximum reported depth of 1524 m; *S. altivelis* adults have a maximum reported depth of 1755 m and are common between 305 and 1755 m (Orr *et al.*, 1998; Lauth *et al.*, 1997). Although the depth ranges of the adults of these species overlap, *S. altivelis* is always more common at greater depths (Hubbs, 1926; Siebenaller and Somero, 1978; Pearcy *et al.*, 1982; Wakefield and Smith, 1990; Lauth *et al.*, 1997). These species have been a sensitive model with which to discern fine-scale adaptations to pressure without the potentially confounding effects of phylogenetic distance or other environmental variables (Siebenaller, 1987; Siebenaller and Somero, 1989).

To test the hypothesis that increased hydrostatic pressure alters guanyl nucleotide binding and thus contributes to the perturbation of G protein-coupled signaling, we have studied the binding of [^{35}S]GTP[S] to the G protein α subunit in response to the A_1 adenosine receptor agonist N^6 (R-phenylisopropyl)adenosine (R-PIA). The binding of [^{35}S]GTP[S] is a direct measure of the GDP-GTP exchange reaction, which is stimulated by R-PIA.

Materials and Methods

Specimens

Demersal adult specimens of *Sebastolobus* were collected using a 40-ft semiballoon otter trawl on cruises of the R/V *Wecoma* off the coast of Oregon. *S. alascanus* was collected at depths of 380 to 415 m; *S. altivelis* was collected from 600 to 1030 m. The collecting sites were at 45° 21.6′ N, 124° 29.6′ W and 45° 30.9′ N, 124° 48.8′ W. Brain tissue was dissected and frozen in liquid N_2 at sea. Tissues were transported to the laboratory on dry ice and maintained at −80°C until used.

Preparation of brain membranes

Brains from several individuals were pooled, and the tissue was homogenized in 100 volumes (volume to weight) of 50 m*M* Tris-HCl, pH 7.4 at 5°C, using a Dounce tissue homogenizer (Pestle A). The sample was centrifuged for 10 min at 27000 × *g* (0–4°C). The pellet was resuspended in 100 volumes of buffer and centrifuged. The pellet was resuspended in 100 volumes of buffer and adenosine deaminase was added to 7.5 units (measured at 25°C) per milliliter. The sample was incubated at 18°C for 30 min, chilled on ice, and centrifuged for 10 min. The pellet was resuspended in 50 volumes of buffer and brought to 7.5 units of adenosine deaminase per milliliter and used in the assays. Adenosine deaminase is included in the incubations to remove endogenous adenosine (see Siebenaller and Murray, 1994c).

Protein determination

Protein was determined using the method of Lowry *et al.* (1951). Samples were solubilized in 0.5 *M* NaOH. Bovine serum albumin (Sigma Chemical Co., St. Louis, MO) was used as the standard.

Binding of [^{35}S]GTP[S] to membranes

The binding of the GTP analog [^{35}S]GTP[S] to membranes was assayed following the methods described by Lorenzen *et al.* (1993) and Wieland and Jakobs (1994). Binding at atmospheric pressure was determined in a volume of 100 μl. The assay mixture contained 50 m*M* Tris HCl, pH 7.4 at the assay temperature of 5°C, 1 m*M* EDTA, 5 m*M* magnesium acetate, 10 μ*M* GDP, 1 m*M* dithiothreitol, 100 m*M* NaCl, 5 mg bovine serum albumin per milliliter, 2.5 units (at 25°C) of adenosine deaminase per milliliter, and approximately 100,000 disintegrations per min (dpm) [^{35}S]GTP[S] (0.3 to 0.5 n*M*). Other additions are as indicated. Approximately 13 to 28 μg of membrane protein was used per tube. The assay was terminated by filtration under vacuum on a Brandel (Gaithersburg, MD) model M-24R cell harvester using Schleicher and Schuell Inc. (Keene, NH) number 32 glass fiber filters. The filters were then rinsed with four 4-ml washes of ice-cold 50 m*M* Tris HCl, pH 7.4 at 5°C, 5 m*M* $MgCl_2$ to remove unbound [^{35}S]GTP[S]. Filter disks were placed into counting vials to which 9 ml of Biocount scintillation fluid (Research Products International Corp., Mount Prospect, IL) was added. Filter-bound radioactivity was determined by scintillation spectrometry (Beckman Instruments, Fullerton, CA, model LS6000IC) following overnight extraction at room temperature. The amount of radioligand bound was less than 10% of the total added in all experiments. Specific binding was defined as total binding minus binding occurring in the presence of 10 μ*M* unlabeled GTP[S]. Nonspecific binding was approximately 1% of the total binding at 0.3 n*M* [^{35}S]GTP[S].

Assays at elevated pressure

The procedures used for assays at elevated pressure were as described by Siebenaller *et al.* (1991). Briefly, samples were sealed in polyethylene tubing and incubated in high-pressure vessels that were kept in a refrigerated water bath at 5°C. The pressure vessels are modeled after those of Zobell and Oppenheimer (1950); the pump and gauges used are as described by Hennessey and Siebenaller (1985). A volume of 200 μl was used for the pressure incubations. A 100-μl aliquot was taken, and the assay was terminated by filtration under vacuum onto a Schleicher and Schuell number 32 glass fiber filter supported on a Hoefer Scientific (San Francisco, CA) single filter holder. The sample was then treated as described above.

All assays were at 5°C to approximate the body temperatures of the two species (Siebenaller and Somero, 1978; Siebenaller and Murray, 1988). Tris was used as the buffer because of the low sensitivity of its pK_a to pressure (Kauzmann *et al.*, 1962). Experiments with both species were conducted in parallel each day. There were no differences in ligand binding between samples incubated in test tubes and samples sealed in tubing and incubated at atmospheric pressure.

Reagents

[^{35}S]guanosine 5′-[γ-thio]triphosphate ([^{35}S]GTP[S], 1332.0 Ci/mmol) was from DuPont NEN (Boston, MA). N^6(R-phenylisopropyl)adenosine (R-PIA) was from Research Biochemicals, Inc. (Natick, MA). Adenosine deaminase (Sigma, type VI) and all other chemicals were from Sigma Chemical Co. (St. Louis, MO). Water was processed with a four-bowl Milli-Q purification system to a resistivity of 10 to 18 MΩ-cm (Millipore, Bedford, MA).

Data analysis

Data were fit using nonlinear regression analyses. [^{35}S]GTP[S] equilibrium binding isotherms were analyzed using AccuFit Saturation-Two Site (Lundeen and Gordon, 1986; Beckman Instruments). Other experiments were analyzed using Prism (version 2.01, GraphPad Software, Inc., San Diego, CA). Comparisons were made using Student's *t* test or analysis of variance with a Tukey-Kramer multiple comparisons test (Sokal and Rohlf, 1995; BIOMstat, version 3.2, Applied Biostatistics, Inc., Setauket, NY, and GraphPad Instat, version 3.00). The *n* values reported represent determinations using different membrane preparations.

Pseudo-first-order association kinetics of the interaction of [^{35}S]GTP[S] and the G proteins were fit to a one-phase exponential association equation:

$$Y = Y_{max}(1 - e^{-kt})$$

where Y is ligand bound, Y_{max} is the maximal binding at the concentration of [^{35}S]GTP[S] used, t is time (min), and k is the observed rate constant. At $t_{1/2}$, when t equals 0.6932/k, $Y = 0.5\ Y_{max}$. The observed rate constant is a function of the on (k_{on}) and off (k_{off}) rates and the concentration of [^{35}S]GTP[S]:

$$k = k_{on}[[^{35}S]GTP[S]] + k_{off}$$

Equilibrium binding was analyzed using the equation:

$$Y = (B_{max} \cdot X)/(K_d + X)$$

where Y represents the specific binding, B_{max} is the maximal specific binding, X is the concentration of free ligand and K_d is the apparent dissociation constant. The K_d is determined by the off and on rates:

$$K_d = k_{off}/k_{on}$$

The fits of the data were also tested against a two-site model, but the two-site model did not statistically improve the fit of the data ($P > 0.05$), based on a partial F test (Hoyer *et al.*, 1984).

Results

High-affinity binding sites for [^{35}S]GTP[S] were characterized in equilibrium saturation experiments at 5°C and atmospheric pressure, using concentrations of [^{35}S]GTP[S] up to 13.5 n*M*. Incubations were carried out for 3 h. The binding capacities and binding affinities of brain membrane preparations of the two species were identical (B_{max}, $P = 0.27$; K_d, $P = 0.88$; Table I).

The IC (inhibitory concentration)$_{50}$ values for GDP were determined at atmospheric pressure using 0.3 n*M* [^{35}S]GTP[S]. The values were 20.66 ± 19.23 μM for *S. alascanus* and 27.60 ± 26.24 μM for *S. altivelis* (mean ± SE, three independent determinations for each species). The IC_{50} values do not differ between the two species ($P > 0.05$).

The EC (effective concentration)$_{50}$ values for R-PIA stimulation of [^{35}S]GTP[S] binding at atmospheric pressure were determined using 0.3 n*M* [^{35}S]GTP[S] in the standard assay mixture. The values were 40.92 ± 17.45 n*M* for *S. alascanus* and 67.04 ± 47.70 n*M* for *S. altivelis* (mean ± SE, three independent determinations for each species). The values do not differ between the species ($P = 0.63$). [^{35}S]GTP[S] binding was maximally stimulated by 3 μM R-PIA. Increased concentrations of R-PIA (10 μM and 100 μM) did not increase the maximal binding of [^{35}S]GTP[S]. For assays of agonist-stimulated [^{35}S]GTP[S] binding, 10 μM R-PIA was used.

The effects of 204 atm pressure on the stimulation of [^{35}S]GTP[S] binding by the A_1 adenosine receptor agonist R-PIA and the muscarinic cholinergic receptor agonist carbamyl choline (carbachol) were examined in 3-h incubations (Fig. 1). Increased pressure inhibited basal [^{35}S]GTP[S] binding about 25% in both species. At 204 atm, both 10 μM RPIA and 100 μM carbachol increased [^{35}S]GTP[S] binding over the basal binding at 204 atm. For these agonists, the percent stimulation was similar in both species.

The time course of the association of [^{35}S]GTP[S] with G proteins was determined at atmospheric pressure (Fig. 2). The time course differed between the *Sebastolobus* species. At atmospheric pressure, brain membrane preparations from *S. altivelis* had $t_{1/2}$ values for binding of [^{35}S]GTP[S] that were significantly higher than for *S. alascanus*. The values determined from the pseudo-first-order association kinetics are given in Table II. The on rate of GTP[S] (k_{on}), calculated from k and the K_d value, is larger in brain membranes of *S. alascanus* than of *S. altivelis* ($2.606 \times 10^7\ M^{-1}\ min^{-1}$ and $1.630 \times 10^7\ M^{-1}\ min^{-1}$, respectively).

In both species the $t_{1/2}$ values were sensitive to increased hydrostatic pressure (Table II). Increased pressure decreased the $t_{1/2}$ values in *S. altivelis* from 58 min at atmospheric pressure to about 36 min. This value was the same at both 204 and 408 atm and was indistinguishable from values seen in *S. alascanus* brain membranes at 1 and 204 atm. In preparations of *S. alascanus* brain membrane, 204 atm did not alter the $t_{1/2}$ value; 408 atm significantly decreased the $t_{1/2}$ value to 13.50 min, less than half the atmospheric pressure value.

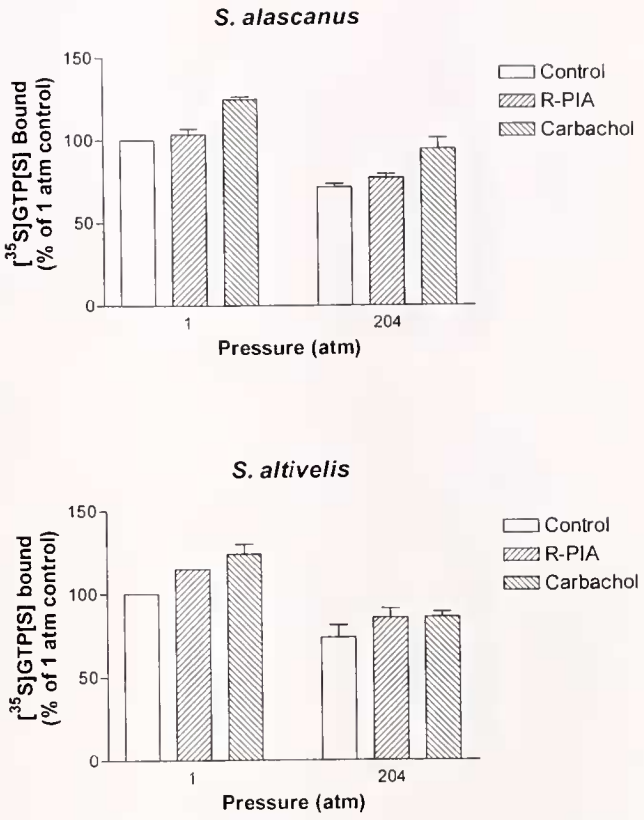

Figure 1. The effects of hydrostatic pressure on the binding of [^{35}S]GTP[S] to brain membrane preparations of *Sebastolobus alascanus* (upper) and *S. altivelis* (lower). [^{35}S]GTP[S] binding is normalized to the atmospheric pressure basal value (no added agonist). The agonists RPIA (10 μM) and carbamyl choline (100 μM) were used. Standard errors are shown. The values represent determinations on five membrane preparations for each species. Increased pressure decreased [^{35}S]GTP[S] binding ($P < 0.05$).

Table I

B_{max} and K_d determinations from [^{35}S]GTP[S] equilibrium binding isotherms at 5°C and atmospheric pressure in the absence of other added guanyl nucleotides

Species	B_{max} (pmol/mg)	K_d (n*M*)
S. alascanus	1.49 ± 0.25	0.41 ± 0.10
S. altivelis	1.15 ± 0.10	0.43 ± 0.07

Mean ± SE of three independent determinations for each species. The values were identical in the two species: B_{max}, $P = 0.27$; K_d, $P = 0.88$.

Discussion

The observation that signal transduction is sensitive to hydrostatic pressure is of general significance for organisms

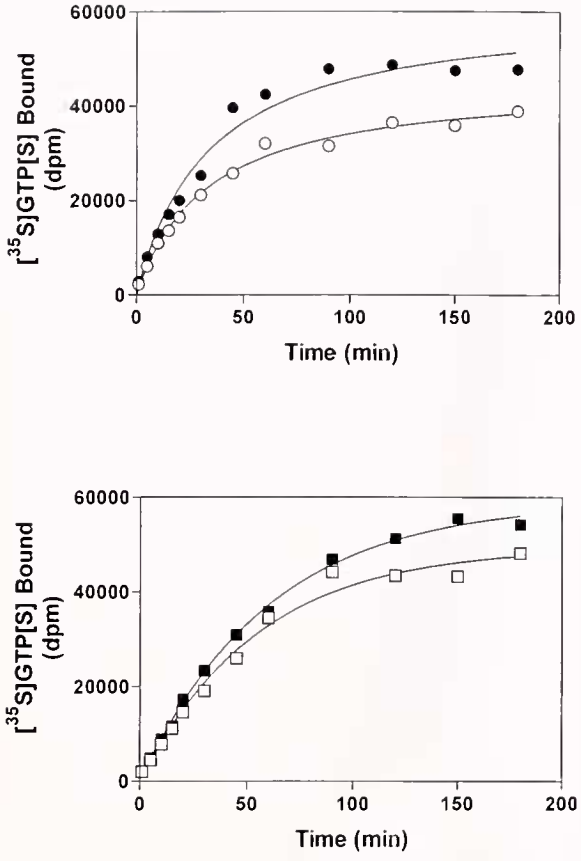

Figure 2. Time course of [^{35}S]GTP[S] binding determined at atmospheric pressure (5°C), in the absence (open symbols) and presence (filled symbols) of 10 μM R-PIA. Upper panel: *Sebastolobus alascanus*. Lower panel: *S. altivelis*. Each point is the mean of three determinations. The standard errors are smaller than the symbols.

colonizing the deep ocean because of the large number of G protein-coupled transmembrane signaling systems (Siebenaller and Murray, 1995). Among the loci of pressure perturbation in these pathways, the intrinsic GTPase activities of the G protein α subunits are affected by increased pressure (Siebenaller and Murray, 1994a). In brain membranes of the congeneric *Sebastolobus* species, increased pressure (340 atm) decreased the apparent K_m of GTP about 10% and increased V_{max} values. Thus, increased pressure stimulates the high-affinity GTPase activity of G protein α subunits and may perturb transmembrane signal transduction by prematurely terminating signaling. The exchange of GTP for GDP is thought to be the rate-limiting step of the GTPase reaction (Higashijima *et al.*, 1987). The present study examined this exchange reaction. Increased pressure can alter this exchange and differentially affects the process in brain membranes of the *Sebastolobus* species.

At atmospheric pressure, the GTP-GDP exchange occurs at different rates in the two species. The $t_{1/2}$ values, which are determined by the on (k_{on}) and off (k_{off}) rates of GTP[S] and the concentration of [^{35}S]GTP[S] used, differ between the two species. The exchange occurs faster in *S. alascanus* brain membranes (Fig. 2, Table I). The on rate in *S. alascanus* is $2.606 \times 10^7\ M^{-1}\ min^{-1}$, in contrast to the k_{on} value of $1.630 \times 10^7\ M^{-1}\ min^{-1}$ in brain membranes of *S. altivelis*. These different rates of GTP binding may reflect differences in the coupling of G proteins and receptors in the *Sebastolobus* species. A number of lines of evidence, including studies of A_1 adenosine receptor agonist binding (Murray and Siebenaller, 1987; Siebenaller and Murray, 1988) and studies using pertussis toxin to probe G protein-receptor interactions (Murray and Siebenaller, 1993; Siebenaller and Murray, 1994b), indicate that in *S. alascanus* brain membranes, a higher fraction of receptors are more closely coupled to G proteins than in *S. altivelis*. The tighter receptor-G protein coupling results in a larger fraction of coupled receptors in *S. alascanus*, which would promote the dissociation of GDP, facilitating [^{35}S]GTP[S] binding to the unliganded guanyl nucleotide binding site on the α subunit of G proteins coupled to receptors. This would be less likely in *S. altivelis* brain membranes because the uncoupled pop-

Table II

Comparison of half-time ($t_{1/2}$) and the binding rate constant (k) of [^{35}S]GTP[S] determined at three pressures and 5°C, in the presence of 10 μM R-PIA

Species	Pressure (atm)		
	1	204	408
S. alascanus			
$t_{1/2}$ (min) ± SE (n)	37.47 ± 2.37 (4)	44.34 ± 7.46 (5)	13.36 ± 2.14 (4)
k (min^{-1})	0.0185	0.0156	0.0519
S. altivelis			
$t_{1/2}$ (min) ±SE (n)	58.06 ± 7.75 (4)	35.52 ± 2.84 (5)	35.75 ± 2.77 (4)
k (min^{-1})	0.0119	0.0195	0.0194

n = the number of determinations on different membrane preparations. Pseudo-first-order association kinetics of the interaction of [^{35}S]GTP[S] and the G proteins were determined from a one-phase exponential association equation. For *S. alascanus*, the $t_{1/2}$ value at 408 atm differs significantly from those at 204 atm ($P < 0.01$) and 1 atm ($P < 0.05$); the values at 1 atm and 204 atm do not differ ($P > 0.05$). For *S. altivelis* the $t_{1/2}$ value at 1 atm differs from those at 204 atm ($P < 0.05$) and 408 atm ($P < 0.05$); the values at 204 atm and 408 atm do not differ ($P > 0.05$).

ulation of G proteins appears to be largely GDP-liganded (Murray and Siebenaller, 1993).

The bulk phospholipid and fatty acid compositions of the brain membranes of the *Sebastolobus* species are the same (Siebenaller *et al.*, 1991). The ordering effects of increased pressure on membrane acyl chain organization (Cossins and Macdonald, 1989) would be expected to also be the same. The difference in coupling efficiency at atmospheric pressure between the species may reflect the need for conformational flexibility and mobility in the membranes at the environmental pressures the species experience (Murray and Siebenaller, 1993). The coupling of receptors to G proteins depends on membrane fluidity (Houslay *et al.*, 1980, 1981; Casadó *et al.*, 1992). The effect of pressure on the time course of GTP binding in the *Sebastolobus* species supports this interpretation.

Increased pressure increases the observed binding constant (decreased $t_{1/2}$) of [^{35}S]GTP[S], and the pressure sensitivities of the observed binding rate constants (k) of the two species differ (Table 1). In *S. altivelis*, the heightened rate of GTP binding may result from increased pressure raising the proportion of G proteins accessible to [^{35}S]GTP[S] binding. Alteration of the localization of signal transduction complexes in the plasma membrane (Huang *et al.*, 1997) or alteration of the interacting pool of subunits (Figler *et al.*, 1997) may contribute to the change in the rate of guanine nucleotide exchange.

Because the $t_{1/2}$ and observed k values depend on the concentration of GTP, values *in vivo* will differ from those reported here. However, it is clear that the pressure effects on the observed binding constant, k (Table II), reflect changes in the binding constants, k_{on} and k_{off}, that are independent of the GTP concentration. Of note is the observation that the k values of the species are similar over the range of *in situ* pressures that the species experience. At the typical habitat pressures of *S. alascanus* (10 to 85 atm), the k value, calculated from the mean of the 1 and 204 atm values, which do not differ, is approximately 0.0171. Over the range of 30.5 to 177.5 atm, the k values for *S. altivelis*, obtained by interpolation, are 0.0128 to 0.0180. These k values were calculated from a plot of the replicates of ln k versus 1 and 204 atm pressure. This assumes that the change in k is due to a pressure-independent volume change. If the pressure effect diminishes at higher pressures, as suggested by the fact that the 204 and 408 atm values are the same in *S. altivelis*, our interpolation underestimates how close the values are for the two species. Nonetheless, at their respective depths of occurrence, the binding rates are more similar than apparent from a comparison at atmospheric pressure. Thus the guanyl nucleotide binding properties of the α subunits appear to be conserved at their habitat pressures.

As seen previously (Siebenaller *et al.*, 1991; Siebenaller and Murray, 1994a), increased pressure decreases the efficacy of agonists (Fig. 1). The maximal binding (B_{max}) values (Table I) for A_1 adenosine receptor agonist stimulated-[^{35}S]GTP[S] binding in *Sebastolobus* brain membranes at 5°C are about 70-fold lower than the B_{max} value (25°C) in bovine brain membranes (Lorenzen *et al.*, 1993). However, the K_d values (Table 1) of the mammalian and teleost species are similar. This observation is consistent with the conservation of binding parameters, and is similar to the pattern observed for enzymes and receptors at their environmental temperatures (*e.g.*, Yancey and Somero, 1978; Yancey and Siebenaller, 1987; Siebenaller and Murray, 1988) and pressures (*e.g.*, Siebenaller, 1987). Maintaining the affinity and selectivity of ligand binding under environmental temperatures and pressures maintains the regulatory and catalytic functions of enzymes (see Hochachka and Somero, 1984) and the selectivity and sensitivity of receptors (Siebenaller and Murray, 1988).

The effects of pressure make it clear that evolutionary adaptation of transmembrane signal transduction complexes is a major challenge for organisms colonizing the deep ocean. The extent of such adaptations for other receptor systems—for example, receptors coupled to G_s—and for receptors in deeper occurring species remain to be determined.

Acknowledgments

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